Final Report

Rewiring a Microbial Chassis to Optimize Electrosynthesis

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Scientific and Technical Objectives

The overall long-term objective of these studies was to develop a chassis microbe for high-rate microbial electrosynthesis, significantly improving the electrical contacts with cathodes and long-range electron transport through cathode biofilms. The research aims were to 1) identify the "bioelectrical plugs" for establishing direct cell-electrode electrical contacts for electron transfer into cells in a potential gram-positive (*Clostridium ljungdahlii*) and gram-negative (*Geobacter sulfurreducens*) chassis for electrosynthesis; 2) determine the "biocircuitry" required to establish long-range electron transport through cathode biofilms; and 3) combine discoveries from Aims #1 and #2 to rewire a chassis for enhanced cathode-to-cell electron transfer.

Approach

The research initially took a genome-scale approach to the elucidation of electron transfer mechanisms in both microorganisms and as more information was obtained became more focused on the (b) (4)

Concise Accomplishments

Significant progress was made toward the long-term objective to develop a chassis microbe for high-rate microbial electrosynthesis. (b) (4)

he studies with *Clostridium ljungdahlii* provided valuable information on its mechanisms for extracellular electron exchange, but suggested it may not be the ideal platform for the design of an electrosynthesis chassis. (b) (4)

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Expanded Overview of Research Accomplished

The research focused on two microorganisms, *Geobacter sulfurreducens* and *Clostridium ljungdahlii*. These two organisms were chosen because they each had attractive features that suggested that they might be a good platform for microbial electrosynthesis. However, the wild-type strains of both microbes had major limitations that had to be overcome with synthetic biology approaches in order to develop a strain that would be an effective microbial electrosynthesis platform. For example, *G. sulfurreducens* was better adapted to extracellular electron exchange. However, *C. ljungdahlii* possessed the Wood-Ljungdahl pathway using carbon dioxide as a terminal electron acceptor, reducing carbon dioxide to acetate, the preferred path for converting carbon dioxide to organic fuels and other commodities.

Development of an Electrosynthesis Platform from *Geobacter sulfurreducens*

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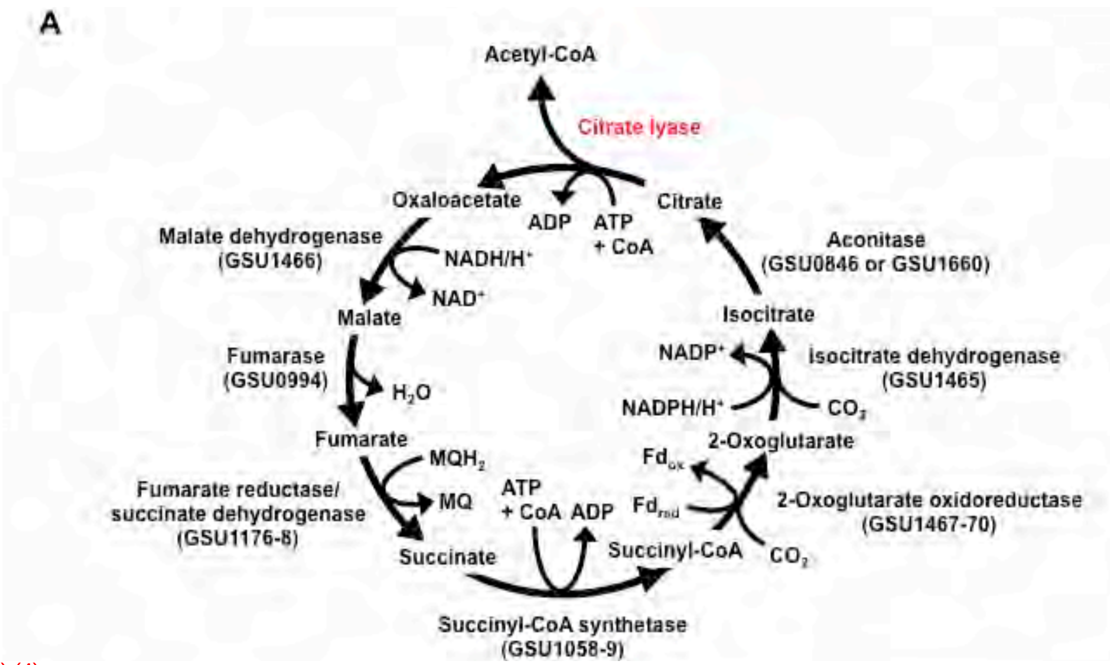
A
Acetyl-CoA
Citrate lyase
Oxaloacetate
ADP
ATP
+ CoA
Citrate
Malate dehydrogenase
(GSU1466)
NADH/H+
NAD+
Aconitase
(GSU0846 or GSU1660)
Malate
Isocitrate
Fumarase
(GSU0994)
H2O
NADP+
Isocitrate dehydrogenase
(GSU1465)
NADPH/H+
CO2
Fumarate
MQH2
2-Oxoglutarate
Fdox
Fumarate reductase/
succinate dehydrogenase
(GSU1176-8)
MQ
ATP
+ CoA
ADP
Fdred
2-Oxoglutarate oxidoreductase
(GSU1467-70)
Succinate
Succinyl-CoA
CO2
Succinyl-CoA synthetase
(GSU1058-9)

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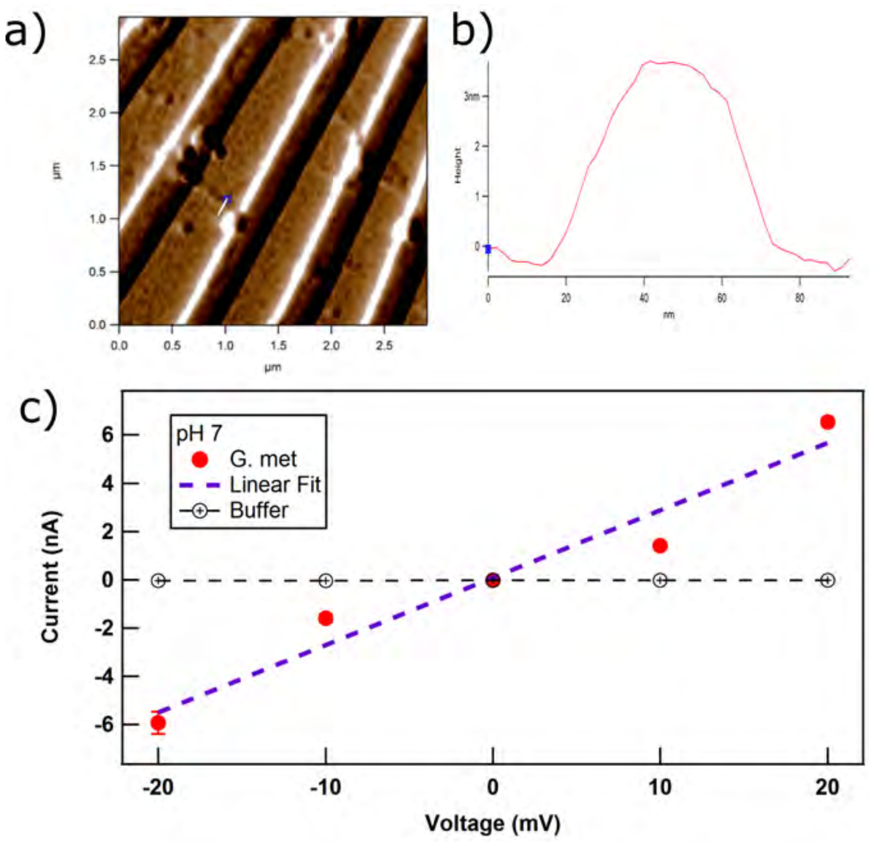
Improving pili conductivity

Previous studies on *G. sulfurreducens* current production demonstrated that high current densities are dependent upon long-range electron transport through biofilms mediated by electrically conductive pili (e-pili). Therefore, the potential of increasing electron transport through biofilms by increasing the conductivity of e-pili was investigated to determine whether this might further improve electrosynthesis.

The first design strategy investigated was the possibility that adding tryptophan to PilA, the pilus monomer, might enhance rates of electron transfer. The gene sequence of PilA was modified so that a tryptophan was substituted for the phenylalanine and the tyrosine at the carboxyl end of the PilA sequence. This modification yielded a strain, designated strain W51W57, which produced pili that were a 2000-fold more conductive than the wild-type pili. Furthermore, the diameter of the W51W57 pili (1.5 nm) was half the diameter of the wild-type pili (Figure 5). This level of conductivity improvement, as well as the ability to make the pili thinner, was completely unexpected. These studies demonstrate the possibility of modifying the properties of pili as a novel electronic material.



<u>Figure 6. Properties of G. metallireducens pili. (a) Atomic force microscope image of individual pili on nano-electrode array. (b) Height measurement with atomic force microscopy. (c) Current-voltage response.</u>

The initial encouraging results with the W51W57 pili led us to question whether there might be pili already naturally produced in the microbial world that are more conductive than the *G. sulfurreducens* pili. An analysis of type IV pilin monomer genes in available microbial genomes demonstrated that the pilin monomer that gives rise to conductive pili in *G. sulfurreducens* has been recently evolved and is primarily found in *Geobacter* and closely related microorganisms. One pilin monomer of particular interest was the pilin of *G. metallireducens*, which contains 9 aromatic amino acids, 3 more than the closely related *G. sulfurreducens*. When the gene for the pilin of *G. metallireducens* was heterologously expressed in *G. sulfurreducens* the pili were 5000-fold more conductive than the native *G. sulfurreducens* e-pili (Figure 6). Unlike the W51W57 pili, the *G. metallireducens* pili have a diameter (3 nm) comparable to that of the *G. sulfurreducens* wild-type pili.

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Elucidating other components of the electron transfer pathway into the cell

A major focus of the research was to determine what other components in *G. sulfurreducens* were important for electron transfer into the cell to donate electrons to respiratory complexes. As detailed in the annual reports, potential electron carriers were identified in multiple strategies for genome-scale transcriptomic analysis. Furthermore, potential targets were identified through analysis of the *G. sulfurreducens* genome and the understanding of the pathways for electron transfer out of the cell. Many gene deletion studies were carried out, often with promising preliminary results. (b) (4)

Introduction of synthetic pathways for carbon dioxide reduction in *G. sulfurreducens*

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<u>Progress Toward Increasing the Ability of *Clostridium ljungdahlii* to Interact with Cathodes</u>
Unlike *G. sulfurreducens, C. ljungdahlii* possess an effective pathway for the reduction of carbon dioxide to multi-carbon organic compounds. Its limitation is the inability to form thick cathode biofilms capable of consuming high current densities.

Initial studies in which the uptake hydrogenase gene was deleted from *C. ljungdahlii* demonstrated that it was directly consuming electrons from the cathode. One hypothesis for electron transfer into gram-positive cells, such as *C. ljungdahlii*, initially proposed by Henry Ehrlich, is that the gram-positive cell walls are electrically conductive. In order to evaluate this hypothesis, cell wall preparations of *C. ljungdahlii* were generated (Figure 12) and the conductivity of the cell wall preparations was evaluated with the two-electrode system that we have previously employed to measure the conductivity of pili and biofilms. (b) (4)

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IMPACT/APPLICATIONS/TRANSISTIONS

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The results also provide additional basic information on the mechanisms for extracellular electron exchange in both *G. sulfurreducens* and *C. ljungdahlii.* (b) (4)

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Additional publications on heterologous expression of conductive pili and further analysis of strain ACL are in preparation.

PATENT APPLICATIONS FILED

Microbial nanowires with increased conductivity and reduced diameters

Microbial strain for electrosynthesis and electrofermentation

Expected additional patent application:
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